correlated with transgene copy number, thereby allowing high levels of expression at high transgene copy numbers.

Introduction of a transgene into the fertilized egg of an animal (e.g., a mammal) is accomplished by any number of standard techniques in transgenic technology. See, e.g., Hogan et al., Manipulating the Mouse Embryo: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986. Most commonly, the transgene is introduced into the embryo by way of microinjection.

Once the transgene is introduced into the egg, the egg is incubated for a short period of time and is then transferred into a pseudopregnant animal of the same species from which the egg was obtained (Hogan et al., supra). In the case of mammals, typically 125 eggs are injected per experiment, approximately two-thirds of which will survive the procedure. Twenty viable eggs are transferred into pseudopregnant mammal, four to ten of which will develop into live pups. Typically, 10-30% of the pups (in the case of mice) carry the transgene.

To identify the transgenic animals of the invention, progeny are examined for the presence of the transgene using standard procedures such as Southern blot hybridization or PCR. Expression of the transgene can also be assessed using Northern blots, Western blots, and immunological assays.

Without further elaboration, it is believed that one skilled in the art can, based on the above disclosure and the description below, utilize the present invention to its fullest extent. The following example is to be construed as merely illustrative of how one skilled in the art can practice the invention and are not limitative of the remainder of the disclosure in any way. Any publications cited in this disclosure are hereby incorporated by reference.

Production of Transgenic Mice

Transgenic mice were produced by microinjection of DNA fragments into the pronuclei of fertilized mouse eggs as described in Brinster et al., Cell 27:223-231, 1981 and Costantini et al., Nature 294:92-94, 1981. Plasmids pHS40-ζ597-GH and pHS40(r-mt 1)-ζ597-GH are described in Zhang et al., Mol Cell Biol 13:2298-2308, 1993. Digestion of these plasmids with EcoRI, NdeI, and ScaI yielded 3.12 kb DNA fragments containing the HS-40 enhancer, the ζ-globin promoter, and the growth hormone (GH) open reading frame. The 3.12 kb DNA fragments were eluted from soft agarose gels, purified, and used for microinjection.

Transgenic founders were identified and their transgene copy number determined by Southern blot analysis of tail DNA. The founders were then bred with nontransgenic C57/B6 mice to establish lines. The morning on which the copulatory pluf was observed was designated 0.5 day postcoital. For analysis of fetal (14.5 days postcoital) and embryonic (9.5 days postcoital) mice, transgenic males were mated to nontransgenic C57/B6 females. Transgenic pups were identified by PCR analysis of fetal mice tails or of embryo DNA. For each identification, duplicate PCR reactions were carried out using one 5′ primer from the ζ-globin promoter region, and two different 3′ primers from the GH region (see below).

A total of 9 founders with wild type HS40-ζGH (wt) and 10 founders with the mutant HS40-ζGH (mt) have been obtained. The copy numbers of integrated fragments in wtHS-40-containing mice vary from 1 to more than 100, as shown in Table 1.

Table 1

Mutant HS-40 Transgene			Wild Type HS-40 Transgene		
Founder line	Copy number	hGH, ng/ml	Founder line	Copy number	hGH, ng/ml
1A*	1	470	1A*	1	36
1B*	1	530	1B*	1	20
1C*	1	1,060	2	2	14
2	2	650	3	3	22
3	3	1,260	5	5	5
8*	8	2,990	10*	10	13
10*	10	3,360	13*	13	187
13*	13	4,650	100A	>100	1,400
15*	15	5,560	100B	>100	30
19*	19	6,490			

In Table 1, the founders for which lines have been established are indicated by an asterisk. Mice with the wtHS-40 transgene were assayed at the age of 5 months except founder 1B, which was evaluated at 9 months old. Mice with the mtHS-40 transgene were assayed at the age of 4 months except founder 15, which was evaluated at 2 months old.

The ζ-globin promoter activities in the founder mice were first measured with a blood GH assay as described in Zhang et al., supra. The levels of human GH in the blood were quantitated with the Allegro hGH radioimmunoassay kit from Nichols Institute. When the concentration of GH in the blood exceeded 50 ng/ml, the samples were first diluted with horse serum in order perform the assay in a linear range.

It was known that the amount of secreted enzyme molecules are good representations of the quantities of